Phenotypic and Ultrastructural Properties of Antigen-Presenting Cells Involved in Contact Sensitization of Normal and UV-Irradiated Mice

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We investigated the surface phenotype and localization of hapten in antigen-presenting cells involved in the induction of contact hypersensitivity or tolerance. Dendritic cells collected 18 h earlier from the draining lymph nodes of mice sensitized with fluorescein isothiocyanate (FITC), which induce contact hypersensitivity upon injection into the footpads of naive mice, stimulated proliferation of lymphocytes from FITC-specific T-cell lines. By immunoelectron microscopy, these cells expressed high amounts of surface Ia molecules but had a negligible amount of FITC on the cell membrane. The majority of the FITC was localized in discrete structures in the cytoplasm, including mitochondria, endocytic vesicles, lysosomes, and cored tubules. In contrast, lymph node cells conjugated with FITC *in vitro* did not stimulate proliferation of FITC-specific T cells and showed a heavy, uniform distribution of FITC throughout the cytoplasm. Draining lymph node cells from mice exposed to ultraviolet (UV) radiation and then sensitized by applying FITC to the UV-irradiated skin, which induce tolerance upon injection into naive mice, induced significantly less proliferation of FITC-specific T cells than draining lymph node cells from unirradiated mice. The differences in activity of these cells, relative to draining lymph node cells from unirradiated, FITC-sensitized mice could not be attributed to a decreased number of Ia^+ dendritic cells in the DLN, decreased surface expression of Ia molecules on these cells, or an alteration in the intracellular localization of hapten. However, a significantly higher percentage of the $FITC^+$ dendritic cells from UV-irradiated mice expressed mac-1, -2, and -3 and F4/80 macrophage markers than did those from unirradiated animals, and fewer cells contained Birbeck granules, suggesting that a different population of Ia^+ antigen-presenting cells may reach the draining lymph nodes of UV-irradiated mice. *Key words: antigen-presenting cells/Langerhans cells/dendritic cells/UV irradiation/immunogold/post-embedding labeling/ultrastructure. J Invest Dermatol 102:928–933, 1994*

Epicutaneous application of reactive haptens to normal human or murine skin induces a contact hypersensitivity (CHS) response, whereas similar application of a hapten to ultraviolet (UV)-irradiated skin induces immunologic tolerance in genetically susceptible individuals [1–3]. To study the cellular events involved in the initiation of these immune responses, we have employed a system in which C3H mice are sensitized by application of fluorescein isothiocyanate (FITC) on shaved, ventral skin, and $FITC^+$ antigen-presenting cells (APCs) are recovered from the draining lymph nodes (DLNs) 18 h later [4]. These APCs are dendritic in morphology, express Ia molecules on their surface [5–8], and are radioresistant [4,7]. Functionally, these cells are APCs, as determined by their ability to induce CHS to FITC when injected into the footpads of syngeneic mice [4,6,7] and to stimulate the proliferation of naive T lymphocytes *in vitro* [5].

In recent studies, we demonstrated that at least some of these cells are derived from epidermal Langerhans cells (LCs), based on the presence of Birbeck granules in the cytoplasm [5,6] and the migration of the cells from skin allografts to the DLNs of nude mice [6]. Thus, these cells acquire hapten in the skin and then migrate to the DLNs, where they form clusters with T lymphocytes [6,9]. We also showed that these cells are capable of internalizing Ia molecules, thereby providing morphologic evidence suggestive of antigen processing [10].

In UV-irradiated mice, $FITC^+$ dendritic cells are also present in the DLNs after epicutaneous sensitization [10,11]. However, injection of these DLN cells into the footpads of normal, syngeneic recipients fails to induce CHS; instead, it induces hapten-specific suppressor T lymphocytes [7]. We are attempting to ascertain the basis for this alteration in antigen-presenting activity *in vivo*. In these studies, we examined the surface phenotype and ultrastructural localization of hapten in $FITC^+$ dendritic cells recovered from the DLNs of FITC-sensitized, UV-irradiated and unirradiated mice and assessed their ability to induce proliferation of cells from FITC-specific T-cell lines. For comparison, we examined the *in vitro* activity and ultrastructural localization of hapten in lymph node cells conjugated *in vitro* with FITC.

MATERIALS AND METHODS

Animals Specific-pathogen-free female C3H/HeNCr(MTV^-) mice were obtained from the Frederick Cancer Research Facility Animal Production Area (Frederick, MD). The mice were housed in a pathogen-free barrier facility, accredited by the American Association for Accreditation of Labora-

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Abbreviations: APC, antigen-presenting cell(s); CHS, contact hypersensitivity; DC, dendritic cell(s); DLN, draining lymph node; GAR, goat anti-rat; GAM, goat anti-mouse; GARab, goat anti-rabbit; SEM, scanning electron microscopy; TEM, transmission electron microscopy.

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tory Animal Care, under conditions that meet the requirements of the National Institutes of Health Guide for Laboratory Animal Care and the United States Department of Agriculture Animal Welfare Act. They were given National Institutes of Health open-formula mouse chow and sterilized water *ad libitum*. Ambient lighting was controlled to provide 12-h light/12-h dark cycles. All experimental procedures were approved by the Institutional Animal Care and Use Committee. Age-matched mice between 10 and 12 weeks of age were used in each experiment.

UV Irradiation This was carried out using a bank of six unfiltered FS40 sunlamps (National Biological Corp., Twinsburg, OH), as described previously [6,7]. The mice were exposed on shaved, ventral skin on four consecutive days for 60 seconds, which resulted in a dose of 400 J/m² per exposure. Unirradiated mice were treated identically, except for the UV irradiation.

DLN Cells Cell suspensions from the pooled lymph nodes of three to five mice were prepared and filtered through nylon mesh [7]. Some of the cells were removed at this stage for use in the *in vitro* proliferation assay and for injection *in vivo* to assess their ability to induce CHS. The remainder were enriched for dendritic cells by centrifugation in Ficoll Hypaque (Pharmacia, Piscataway, NJ) at 1750 rpm (600 × g) for 30 min at 4°C.

DLN cells conjugated *in vitro* with FITC were incubated with 0.25% (w/v) FITC in phospate-buffered saline (PBS), pH 7.4 at 37°C for 30 min. After being washed three times, the samples were used in the *in vitro* proliferation assay or were fixed and processed for postembedding labeling. In addition, DLN of mice from each treatment group were cut into small pieces and fixed directly for postembedding labeling of intracellular FITC.

Contact Sensitization Several hours after the last UV exposure, mice were sensitized on shaved abdominal skin with 0.4 ml of a solution containing 0.5% (w/v) FITC (Aldrich Chemical Co., Milwaukee, WI) in solvent composed of equal volumes of acetone and dibutyl phthalate [4] or with 0.05 ml of 0.3% (v/v), 2,4-dinitrofluorobenzene (DNFB) in acetone. The mice were killed 18 h later and their DLN were removed. To assess the antigen-presenting activity of these cells, 1×10^6 DLN cells were injected subcutaneously into each hind footpad of a group of five normal mice. The recipients were challenged 6 d later by painting 5 μl of 0.5% FITC or 0.3% DNFB on each ear surface; ear swelling was determined by measuring the thickness of the ears with a spring-loaded micrometer (Mitutoyo, Tokyo, Japan) before and 24 h after challenge [6,7].

Derivation and Maintenance of FITC-Specific T-Cell Lines (T_{FITC}) C3H/HeN mice were sensitized with FITC, as described above, and 5–14 d later they were boosted by a second application of FITC. Eighteen hours later, the mice were killed, and the DLNs were removed and a cell suspension was prepared. The DLN cells were cultured in T25 plastic tissue culture flasks (Becton-Dickinson Labware, Lincoln Park, NJ) in RPM 1640 medium containing 10% fetal calf serum (FCS), 4 mM L-glutamine, penicillin/streptomycin, 10% Con-A supernatant, 10 units/ml recombinant human IL-2, and 5×10^{-5} M β-mercaptoethanol. The cultures were fed weekly with γ-irradiated (2500 rads from a Cs source) DLN cells from mice that had been epicutaneously sensitized with 0.5% FITC 18 h earlier. Every 3 weeks, dead cells were removed by separation on Ficoll-Hypaque gradients, and fresh stimulator cells were added.

***In Vitro* Proliferation Assay** DLN cells from C3H/HeN or C57/BL6 mice epicutaneously sensitized with FITC or DNFB were suspended in RPMI 1640 medium containing 10% FCS, 4 mM glutamine, 5×10^{-5} M β-mercaptoethanol, penicillin/streptomycin, and γ-irradiated (2500 rads). These stimulator cells were cultured in 96-well round-bottom microtiter plates with 1 to 5×10^4 Ficoll-purified T_{FITC} for 48 h at 37°C in a 5% CO_2/95% air atmosphere. During the final 6 h of incubation, 1 μCi [³H]thymidine was added to each well. The cultures were harvested and counted in a scintillation counter. Values expressed are means ± standard error of the counts per minute of triplicate cultures.

Antibodies For detection of surface markers, monoclonal rat anti-Thy1.2 and mouse anti-I^k were obtained from Becton-Dickinson Immunocytometry Systems (Mountain View, CA). Hybridoma cells producing the antibody rat anti-F4/80 were the gift of Dr. David Hume (University of Queensland, Brisbane, Australia). Monoclonal rat anti-mac-1, -mac-2, and -mac-3 were supernatants from hybridomas purchased from the American Type Culture Collection (Rockville, MD). Gold-labeled goat anti-rat (GAR), goat anti-mouse (GAM), and goat anti-rabbit (GARab) secondary antibodies were obtained from Janssen Life Sciences (Amersham Corp., Arlington Heights, IL). Rabbit anti-FITC was obtained from Molecular Probes (Eugene, OR).

Combined Immunogold and Fluorescence Microscopy Dendritic cells (DCs) were fixed lightly with 2% paraformaldehyde in PBS for 10 min, washed, and resuspended in RPMI 1640 medium. The cells were allowed to adhere to poly-L-lysine-coated coverslips for 1 h and incubated with PBS containing 1% normal goat serum, 1% (BSA), and 1% normal horse serum for 20 min at room temperature. Immunolabeling was accomplished by sequential incubation of cells with a primary antibody and the appropriate gold-labeled secondary antibody for 1 h each. The samples were washed with PBS, refixed with 4% paraformaldehyde in PBS for 10 min, washed with distilled water three times, and treated with Silver Intense (Amersham Corp.) for 7 min. The samples were examined sequentially for both gold labeling and FITC fluorescence. The entire coverslip was scanned in a grid pattern to ensure that each cell was counted only once.

Table I. Proliferation of T_{FITC} Cell Lines in Response to FITC-Bearing Dendritic Cells

Source of Purified APCs[a]	Number of APCs	Number of T_{FITC} Cells[b]	Mean cpm ± SEM[c]
Experiment 1[d]			
None	0	1×10^4	203 ± 15
NR-FITC[e]	1×10^5	0	87 ± 19
NR-FITC	1×10^5	1×10^4	7,771 ± 1,015
FITC *in vitro*[f]	1×10^5	0	157 ± 36
FITC *in vitro*	1×10^5	1×10^4	791 ± 136[g]
C57/BL6-FITC[h]	1×10^5	0	85 ± 3
C57BL/6-FITC	1×10^5	1×10^4	1,680 ± 242[g]
NR-DNFB[e]	1×10^5	0	80 ± 20
NR-DNFB	1×10^5	1×10^4	959 ± 47[g]
Experiment 2[d]			
None		1×10^4	154 ± 43
NR-FITC	5×10^4	None	964 ± 148
NR-FITC	5×10^4	1×10^4	4,458 ± 246
UV-FITC[j]	5×10^4	None	677 ± 99
UV-FITC	5×10^4	1×10^4	1,439 ± 145[i]
Experiment 3[k]			
None	0	1×10^4	497 ± 44
NR-FITC	5×10^4	None	147 ± 41
NR-FITC	5×10^4	1×10^4	25,928 ± 1,194
UV-FITC	5×10^4	None	296 ± 62
UV-FITC	5×10^4	1×10^4	15,359 ± 958[i]

[a] Dendritic cells were purified on 18% metrizamide gradients and represented 10–30% of the total population after enrichment. Cells were γ-irradiated (2500 rads) before assay.

[b] FITC-specific T-cell lines $T_{4/28}$ or $T_{3/05}$ were purified from maintainance cultures on Ficoll-Hypaque gradients. The cell lines were used for these experiments 5–7 d after the last restimulation with γ-irradiated (2500 rads) DLN cells from C3H mice epicutaneously sensitized with FITC.

[c] Cells cultured 48 h in 96-well plates. 1 μCi [³H]thymidine added to each well for the final 6 h of incubation. Data represent the mean ± standard error of triplicate wells.

[d] T-cell line $T_{4/28}$.

[e] C3H/HeN ($H\text{-}2^k$) mice epicutaneously sensitized with hapten 18 h earlier.

[f] C3H/HeN lymph node cells covalently coupled with FITC *in vitro*.

[g] $p < 0.005$ versus NR-FITC APC + T_{FITC} group.

[h] C57BL/6 ($H\text{-}2^b$) mice epicutaneously sensitized with hapten 18 h earlier.

[i] $p < 0.005$ versus NR-FITC APC + T_{FITC} matching control. Statistical analyses were performed by ANOVA.

[j] C3H/HeN mice were exposed to 2 kJ/m² UVB radiation 3 d before epicutaneous sensitization with FITC; DCs were collected 18 h after sensitization.

[k] T-cell line $T_{3/05}$.

Preparation of Samples for Transmission Electron Microscopy (TEM) For pre-embedding labeling, enriched DC suspensions were incubated with anti-Thy-1.2 or anti-I^k monoclonal antibody or rabbit anti-FITC for 1 h at 4°C as described previously [10]. After being washed three times, the samples were incubated with a 1:4 dilution of the appropriate gold-labeled secondary antibody for 1 h at 4°C. After three more washes, the samples were fixed in a solution of 3% glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, for 1 h at 21°C and processed for TEM [6].

For postembedding labeling, samples were fixed in a solution of 4% paraformaldehyde plus 0.25% glutaraldehyde at 21°C for 2 h, washed three times with PBS, and left overnight in PBS at 4°C. The samples were washed with fresh PBS, dehydrated in a graded series of ethanol at −20°C, and then embedded in Lowicryl K4M (Polysciences, Inc., Warrington, PA) at −40°C. The samples were polymerized under UV light at −40°C for 24 h and at 21°C for 3 d. Ultrathin sections were cut in an LKB Nova Ultramicrotome and picked up on formvar-coated nickel grids. The sections were etched with freshly prepared 1% sodium periodate for 10 min and then washed with PBS three times. The grids were floated on drops of PBS

Table II. Reactivity of FITC$^+$ Dendritic Cells with Various Macrophage Markers[a]

Treatment of DLN Donors	Primary Antibody	Number of FITC$^+$ DCs Counted	Number of FITC$^+$ DCs with Gold Label	Percent	$p <$
FITC	mac-1	47	8	17	
	mac-2	33	1	3	
	mac-3	30	1	3	
	F4/80	42	19	45	
UV-FITC	mac-1	41	26	63	0.001
	mac-2	32	8	25	0.01
	mac-3	30	7	23	0.01
	F4/80	17	15	88	0.01

[a] DLN cells were enriched for DCs and immunogold labeled. Cells were scored by identifying individual FITC$^+$ cells by epifluorescence microscopy and examining them under bright field optics to determine the presence of gold label.

containing 2% BSA for 20 min and then incubated with a 1:20 dilution of rabbit anti-FITC antibody for 2 h at 21°C. They were washed five times with PBS, incubated with PBS/BSA for 5 min, and transferred to a 1:4 dilution of gold-labeled GARab antibody for 2 h at 21°C. The grids were washed five times and counterstained with uranyl acetate followed by lead citrate in an LKB Ultrastainer and examined under a JEOL 1200 EX transmission electron microscope at 100 kV.

Postembedding Immunogold Labeling on Epon Sections In an attempt to identify the cellular organelles that showed distinct anti-FITC labeling in the Lowicryl-embedded samples, we performed the immunolabeling procedure according to the method described by Stossel *et al.* [12] for detecting acidic organelles in isolated Langerhans cells. Briefly, cell pellets of the enriched population of DCs were fixed in Karnovsky's half-strength formaldehyde/glutaraldehyde fixative for 1 h, washed with 0.1 M cacodylate buffer, postfixed with 1% aqueous osmium tetroxide for 1 h, washed with distilled water, and *en bloc* stained with 1% aqueous uranyl acetate for 30 min. The pellets were dehydrated in a graded series of ethanols, infiltrated in a graded series of propylene oxide/Epon mixtures, embedded in Epon 812, and polymerized at 60°C for 24 h. Ultrathin Epon sections were mounted on nickel grids, etched with 2% sodium periodate for 30 min, and rinsed. Immunogold labeling was performed by sequential incubation of grids with the following reagents: a) 1:20 dilution of rabbit anti-FITC for 2 h at room temperature; b) 1:10 dilution of gold-labeled recombinant Protein A/G (12 nm gold, electron microscopy (EM) grade) (Jackson Immunoresearch Laboratories, Inc., West Grove, PA) for 1 h. All immunoreagents were diluted with Tris-buffered saline (TBS) containing 1% BSA plus 1% normal goat serum and 0.1% Tween-20. After incubation, grids were counterstained with uranyl acetate and lead citrate as described above.

RESULTS

Activity of DLN Cells In previous studies we demonstrated that DLN cells from FITC-sensitized mice induced CHS when injected subcutaneously into the footpad of normal, syngeneic recipients [6,7], whereas those from UV-irradiated mice induced specific tolerance [7]. This finding was confirmed in these studies using some of the same populations of cells as those used for phenotypic and ultrastructural analysis (not shown). We have also shown previously that intra-footpad injection of lymph node cells conjugated *in vitro* with FITC induces neither CHS nor tolerance [13]. The ability of these three cell populations to activate the proliferation of T lymphocytes *in vitro* was assessed using syngeneic, FITC-specific T-cell lines. As shown in Table I, proliferation of the T cells was induced by γ-irradiated DLN cells from FITC-sensitized mice; proliferation was antigen specific and H-2 restricted (experiment 1). DLN cells from UV-irradiated, FITC-sensitized mice also induced proliferation, but to a significantly lesser extent than those from unirradiated mice (experiments 2 and 3). Lymph node cells conjugated *in vitro* with FITC failed to stimulate proliferation of the FITC-specific T cells (experiment 1).

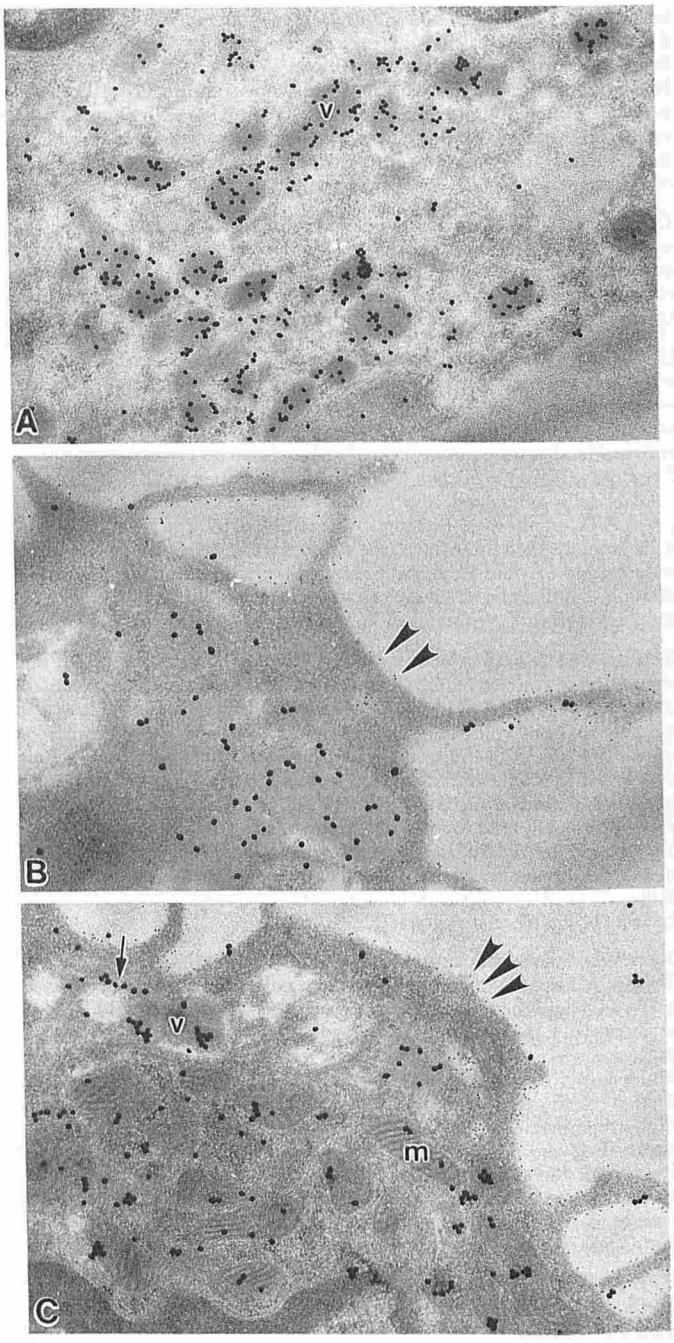

Figure 1. Postembedding labeling (Lowicryl embedding) of dendritic cells from FITC-sensitized mice. The cells show major reactivity with anti-FITC in the cytoplasm *(A)*, and gold particles (30-nm diameter) are seen associated with mitochondria and vesicles *(A–C)*. Very few 30-nm gold particles are seen along the plasma membrane *(B,C)*. Gold particles were sometimes seen in a linear array in the cytoplasm *(arrow, C)*. Ia$^+$ FITC$^+$ dendritic cells were identified by the surface labeling with anti-Ia (*arrowheads,* 10 nm gold) and cytoplasmic labeling with anti-FITC (30 nm gold) *(B,C)*.

Phenotype of DCs from UV-Irradiated and Unirradiated Mice Comparing the populations of enriched DCs from FITC-sensitized, UV-irradiated or unirradiated mice by light and epifluorescence microscopy revealed that the same or a greater number of DCs were present in the preparations obtained from UV-irradiated mice, compared to unirradiated mice, and that the proportion of FITC$^+$ cells, which ranged from 56% to 74% in different experiments, was the same in both groups (not shown). Labeling the

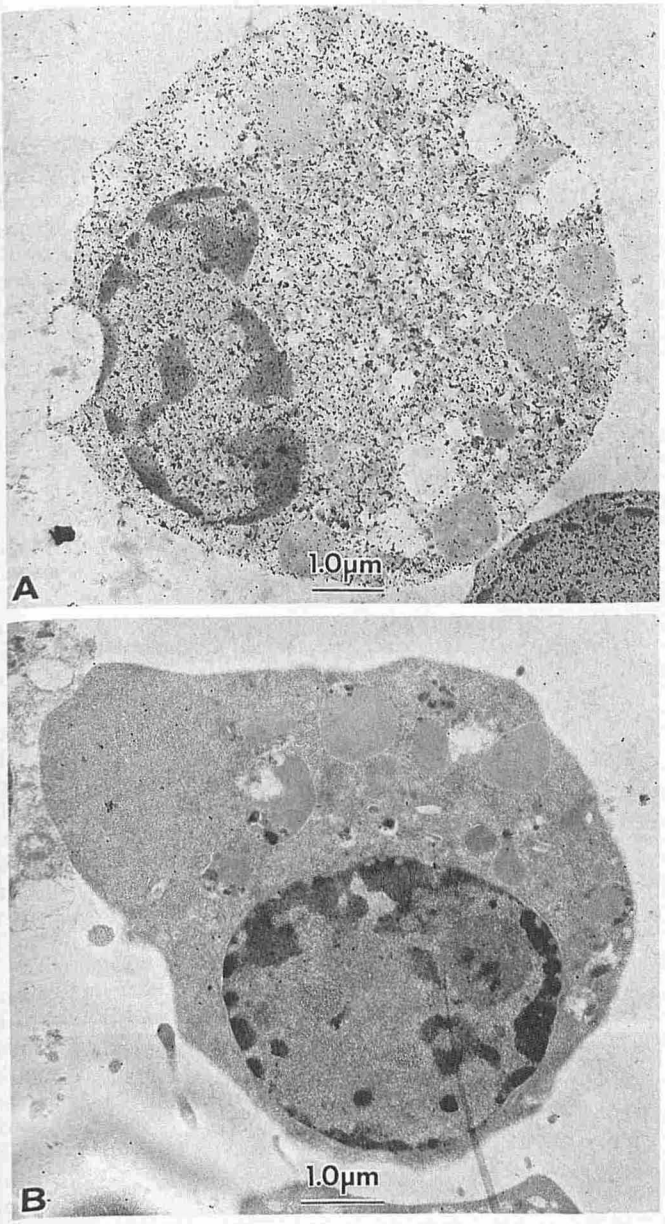

Figure 2. Lymph node cells conjugated *in vitro* with FITC, embedded in Lowicryl, and immunolabeled with anti-FITC antibody. The cells exhibit a uniform distribution of gold particles except in vacuoles, where only a few gold particles are seen *(A)*. A cell treated with an irrelevant antibody plus the gold-labeled second antibody is shown in *B*.

preparations with anti-Ia antibody before fixation produced prominent surface labeling of virtually all DCs when examined by light microscopy, scanning electron microscopy (SEM), and TEM; the cells were uniformly negative for Thy1.2 (not shown). No differences were detected between preparations obtained from UV-irradiated or unirradiated mice.

To further characterize the DCs, individual FITC$^+$ DCs were identified using epifluorescence microscopy and examined under bright-field optics for gold-labeled surface markers for mac-1,-2, and -3 and F4/80. As shown in Table II, a significantly greater proportion of the FITC$^+$ cells from the UV-irradiated mice expressed these markers compared with DCs from unirradiated mice.

In contrast to the prominent surface expression of Ia molecules, little or no surface labeling could be detected with anti-FITC antibody using TEM. Because approximately 55–75% of the cells in the enriched DC preparation were fluorescent by epifluorescence microscopy, and because the anti-FITC antibody could detect surface FITC on *in vitro*-labeled cells analyzed by TEM, this suggested that the hapten had been internalized and was not detectable by prefixation labeling.

Table III. Identification of FITC$^+$, Ia$^+$ Dendritic Cells in DLNs of FITC-Sensitized, UV-Irradiated, and Unirradiated Mice Using TEM[a]

Treatment of DLN Donors	Total Number of Ia$^+$ DCs Present	Number of Ia$^+$ FITC$^+$ DCs	Percent
FITC	25	9	36
UV, FITC	51	21	41

[a] Five nonserial sections were examined and photographed. Ia was detected on the cell surface by pre-embedding labeling with anti-Ia and 10 nm gold-labeled secondary antibody; nearly all DCs were Ia$^+$. Intracellular FITC was detected by postembedding labeling with anti-FITC and 30-nm gold-labeled secondary antibody. In DCs that were Ia$^+$, 30-nm gold particles were counted, and cells in which the cytoplasm-to-nucleus ratio of gold particles was >2 were considered FITC$^+$.

Postembedding Labeling of DCs with Anti-FITC To visualize FITC inside the DCs, ultrathin sections of Lowicryl-embedded samples were treated with anti-FITC antibodies. As shown in Fig 1, numerous gold particles were present within the DCs, and very few were associated with the cell membrane. Nondendritic cells in the preparations showed only background labeling (not shown). The gold label was preferentially localized in the cytoplasm, mainly in association with vesicles and mitochondria (Fig 1); no label was observed in the centrosome area, and only background amounts of label were present in the nucleus. The same pattern of intracellular labeling was observed in DCs obtained from UV-irradiated and unirradiated, FITC-sensitized mice.

The localization of intracellular FITC in discrete cytoplasmic structures in the DCs obtained from *in vivo* sensitized mice contrasted dramatically with the distribution of FITC in cells conjugated *in vitro* with FITC. *In vitro* incubation of cells with hapten resulted in a uniform distribution of gold particles throughout the entire cell, with the exception of vacuoles, which contained few gold particles (Fig 2*A*). Cells incubated with an unrelated rabbit antiserum and the gold-labeled secondary antibody showed only background labeling (Figure 2*B*).

When DCs were labeled *in situ* in tissue sections to examine the distribution of hapten, the label was present inside DCs located in the paracortical area of the lymph node. The labeling pattern was similar to that of the partially purified DC suspensions, in that the majority of the label was associated with mitochondria and vesicles. Adjacent lymphocytes contained only background amounts of label (not shown).

Combined Pre- and Postembedding Labeling of DC Ia molecules were no longer detectable on or in cells that were labeled after the embedding procedure. This problem precluded examining the colocalization of Ia and hapten molecules inside the DCs using this technique. However, combining the pre- and postembedding labeling procedures allowed us to identify DCs that were positive for both Ia and FITC. As illustrated in Fig 1*B,C,* all FITC$^+$ DCs expressed surface Ia molecules; however, not all of the Ia$^+$ DCs contained intracellular FITC. We attempted to determine the frequency of Ia$^+$ FITC$^+$ cells by photographing five nonserial sections of the cell preparation. Table III indicates that, using this approach, 36% of the Ia$^+$ DCs from unirradiated mice were also FITC$^+$. The DLN cell preparation from UV-FITC mice contained more Ia$^+$ DCs, but the proportion of Ia$^+$ FITC$^+$ cells was nearly identical to that of the cells from unirradiated mice. This finding is consistent with the results of the light microscopic study, although the percentage of FITC$^+$ DCs was higher using light microscopy.

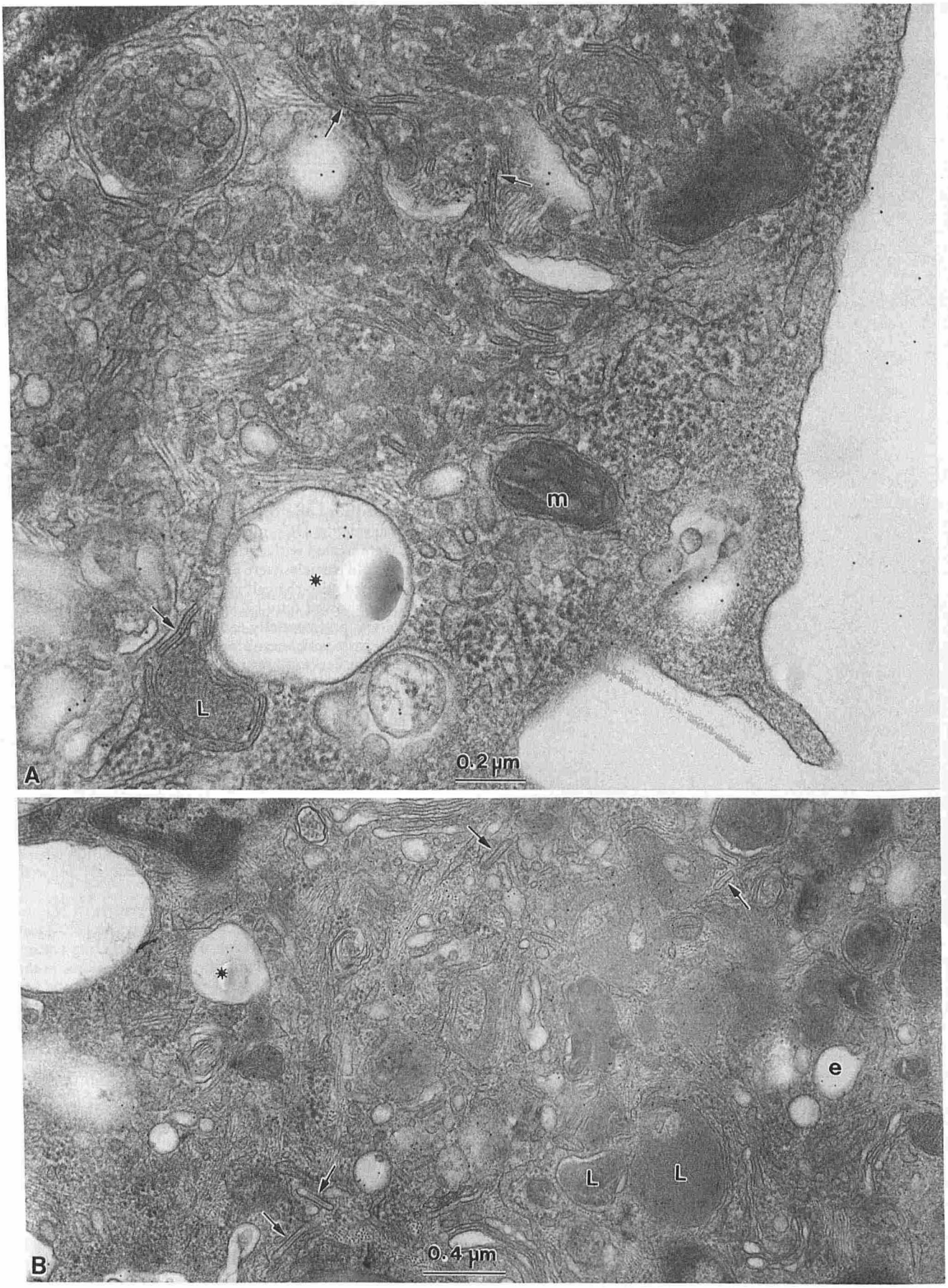

Figure 3. Epon-embedded DLN dendritic cell from UV-irradiated FITC-sensitized mice. *A)* Postembedding labeling with anti-FITC shows labeling of endosome-like structures *(asterisk)*, mitochondria (m), cored tubules *(arrow)*, and lysosomes (L). Surface labeling is very sparse. *B)* DC shows labeling of electron-dense lysosomes (L) and a late endosome (e). Other endosome-like structures *(asterisk)* are also labeled, but Birbeck granules *(arrows)* are not labeled.

Localization of FITC in Epon-Embedded Samples The Lowicryl postembedding procedure preserves the antigenicity of the FITC, but because of the light fixation procedure, there is very little contrast in the intracellular structures in the specimen. Thus, it was difficult to identify the vesicles and other cytoplasmic organelles with certainty. Marked improvement in the ultrastructural morphology was noted when we performed the postembedding labeling procedure on Epon-embedded specimens and used gold-labeled, recombinant protein A/G [14] to detect the anti-FITC antibody. As illustrated in Fig 3, the label was localized in endosome-like structures, mitochondria, cored tubules, lysosomes, and large, slightly electron-dense vesicles in DCs from both UV-irradiated and unirradiated mice. Some gold particles were also adjacent to microfilaments. Only background staining was present in the nucleus. Few Birbeck granules were detectable in the DCs from UV-FITC mice; no gold particles were associated with these structures in DCs from either UV-irradiated or unirradiated mice.

DISCUSSION

In these studies, we have compared some of the phenotypic and ultrastructural characteristics of DCs from the DLNs of UV-irradiated and unirradiated mice sensitized epicutaneously with FITC. These cell populations differ dramatically in their ability to induce an immune response to FITC *in vivo*. Injection of DLN cells from UV-irradiated mice into the footpad of normal, syngeneic recipients induces hapten-specific, suppressor T lymphocytes, whereas those from unirradiated mice induce CHS to FITC [7]. When used to stimulate the proliferation of FITC-specific T lymphocytes *in vitro,* DLN cells from the UV-irradiated mice were less effective than DLN cells from unirradiated mice, but they were still capable of inducing a significant response. Because the FITC-specific T-cell lines are heterogeneous and contain both $CD4^+$ and $CD8^+$ cells (F. Strickland, manuscript in preparation), it is possible that different subpopulations of T cells are responding to the DCs derived from UV-irradiated and unirradiated donors. Studies with cloned T-cell lines should resolve this question.

Comparison of the $FITC^+$ DC populations from UV-irradiated and unirradiated mice with regard to number and surface markers also revealed several differences. A significantly greater proportion of $FITC^+$ DCs from the UV-irradiated mice expressed the macrophage markers mac-1, -2, and -3, and F4/80, and sometimes there were more Ia^+ DCs in the DLN of the UV-irradiated mice. Ultrastructurally, fewer DCs from the UV-irradiated mice contained identifiable Birbeck granules, as we reported previously [11]. These findings suggest that a significant proportion of the $FITC^+$ dendritic cells in the DLNs of the UV-irradiated mice may be derived from inflammatory cells that infiltrate the skin in response to UV radiation. This result is consistent with the studies of Cooper and colleagues [15], suggesting that non-Langerhans cells that infiltrate the skin after UV irradiation are responsible for tolerance induction.

The ultrastructural location of the FITC in and on DCs was examined using gold-labeled antibody. The vast majority of the hapten was localized in subcellular organelles within the DCs, and very little was detectable on the cell surface. The location of hapten in endosome-like vesicles and lysosomes is similar to that of a marker of acidic cytoplasmic organelles in freshly isolated Langerhans cells [12]. However, there are several differences between our results with *in vivo* sensitized DLN cells and those with unsensitized epidermal Langerhans cells. We found no evidence of gold particles associated with Birbeck granules, and we did find label in cored tubules and mitochondria. These differences may reflect the state of activation of antigen-stimulated cells or the fact that intracellular hapten is not restricted solely to acidic compartments. Comparison of DCs from UV-irradiated and unirradiated mice failed to reveal any differences in the subcellular localization of FITC.

In contrast, lymph node cells conjugated *in vitro* with FITC exhibited heavy labeling, with a fairly uniform intracellular distribution of FITC. The lack of intracellular hapten localization correlated with a lack of antigen-presenting function of these cells *in vivo* [13] and *in vitro;* however, the lack of activity could be due to many other factors, such as a lower frequency of Ia^+ DCs in the lymph nodes from unsensitized mice.

Although the ultrastructural comparison between DCs from UV-irradiated and unirradiated mice did not reveal a difference in Ia expression or hapten localization that could account for the dramatic difference in their activity, several conclusions may be drawn from these studies. First, although UV irradiation of murine and human Langerhans cells can reduce the expression of class II major histocompatability complex molecules [16,17], decreased surface expression of Ia could not account for the altered antigen-presenting activity of DCs from UV-irradiated mice in our experiments. Second, the failure of DLN cells from UV-irradiated mice to induce CHS *in vivo* [7] does not result from a decreased number of $FITC^+$, Ia^+ DCs in the DLNs. Third, the DLNs of UV-irradiated mice contain a higher proportion of $FITC^+$ DCs expressing markers found on inflammatory macrophages and a lower proportion of $FITC^+$ DCs containing Birbeck granules, suggesting that there may be a different population of FITC-bearing, antigen-presenting cells that reaches the DLNs in UV-irradiated mice. This difference in the population of $FITC^+$ DCs could account for its altered antigen-presenting activity. Studies on the activity of $FITC^+$ cell populations purified by flow cytometry are currently in progress and should provide a direct test of this possibility.

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